REMARKS

In view of the above amendments and the following remarks, reconsideration of the outstanding office action is respectfully requested.

Claims 1, 3, 19, 28-29, 46, 48, and 62 have been amended. No new matter has been added. Claim 4 has been canceled. Claims 1-3 and 5-62 are currently pending.

The transgenic process is an economically attractive method of producing large amounts of human therapeutic proteins. This involves the creation of genetically altered animals and plants such that the desired heterologous protein is recoverable in their milk, eggs, fruit, etc. A DNA construct that encodes the target human protein, is inserted into a goat cell line by transfection. The transfected transgenic cell is then fused with an enucleated oocyte by electrofusion. After 24-48 hours in culture, the embryo is transferred to a surrogate mother. The putative transgenic animals are identified by screening the offspring for the transgene. After the selected females mature, they are bred and the milk produced after gestation is tested for protein expression.

Transgenic versions of therapeutic proteins, implicated in chronic diseases, like human monoclonal antibodies, tissue plasminogen activator, antithrombin III, and human lactoferrin are in various stages of FDA approval. These products are being developed as treatments for arthiritis, HIV/AIDS, cancer, and autoimmune diseases. One limitation of the transgenic process is the long lag-time between cloning and production (~ 18 months). However, recent developments have purportedly cut this time in half.

The complexity of milk combined with the low concentration of target protein complicates the recovery process from transgenic milk. Whole milk consists of more than 100,000 different molecules dispersed in three phases namely, lipid, casein, and whey and heterologous recombinant proteins can be overproduced in the range of 0.2 to 1 wt. %. Traditional methods used by the dairy industry to isolate proteins from milk involving pasteurization followed by enzymatic coagulation or acid precipitation at pH 4.6 (pI of casein) are unsuitable for the recovery of heterologous proteins because they can be temperature and pH sensitive. Additionally, the coagulation process can trap a large amount of the target protein within the casein pellets resulting in poor yields. Transgenic milk is neither pasteurized nor homogenized in order to prevent damage and loss of the target heterologous proteins. In non-homogenized transgenic milk, the liquid fat droplets, ranging from 0.1 to 20 μm in diameter, are encased by a 8 to 10 nm thick membrane called the native fat globule membrane (FGM). The FGM is composed of phospholipids and proteins and is

characterized by a very low interfacial surface tension, 1 to 2.5 mN/m, between the fat globules and the serum phase. This prevents the globules from flocculation and from enzymatic degradation. Homogenization breaks up the fat globules causing disruption of the native FGM, which allows serum proteins and casein micelles to freely adsorb onto the exposed fat globules. This results in loss of heterologous proteins through adsorption. The latter effect is expected to reduce the yield of target protein and the former effect increases membrane fouling because of a lower value of back-transport due to shear or inertial lift of small fat globules. The casein micelle is a roughly spherical, fairly swollen particle of 0.1 to 0.3 μm diameter with a hairy outer layer. The hairy layer is comprised of C-terminal ends of κ-casein. This prevents further aggregation of micelles and flocculation by steric and electrostatic repulsion at pH values higher than 4.6, the pI of casein. Thus, at the physiological pH of milk, 6.4-6.6, the casein micelles predominantly exist as distinct particles of a size range comparable to the mean pore size (0.1 μm) of the poly(ether sulfone) microfiltration membrane used here. This is expected to result in a low shear-induced diffusion coefficient as well as fouling by pore blockage and cake formation, especially at low shear rates. Fat globules and casein micelles are retained in whole milk microfiltration, whereas the product protein permeates through the membrane along with the whey proteins, minerals, and sugars. This is corroborated by polyacrylamide gel electrophoresis studies of permeate samples of milk clarified by microfiltration with a 0.2 μm average pore size ceramic membrane which indicate negligible casein transmission through the membrane.

The present invention is directed to an improved procedure for recovering target molecules from polydisperse liquids, including recovering components from milk.

The objection to claim 3 for depending upon itself is traversed in view of the amendment above.

The rejection of claims 28 and 29 under 35 U.S.C. § 112 (second para.) for indefiniteness is respectfully traversed in view of the amendments above.

The rejection of claims 1-2, 4, 7, 8, 11-17, 28-29, 31, 34-35, and 38-44 under 35 U.S.C. § 102(b) as anticipated by U.S. Patent No. 6,875,459 to Kopf et al. ("Kopf") is respectfully traversed.

Kopf teaches a method of filtering various components and subcomponents of milk using cross flow filtration devices, which can include, *inter alia*, microfiltration and ultrafiltration systems; the use of multiple filter sheets in an operative stacked arrangement is disclosed. Filtration membranes can be in any format, including hollow fibers, spiral, tubular

and ceramic. Kopf also teaches that filtration should be performed with cross flow and channel velocities between 0.5 and 2.5m/sec and a flow velocity between 32-40 lm^2h depending on the membrane. Kopf achieves retentate yields of 100% for some proteins and permeate protein concentrations of greater than 7%.

Kopf fails to teach recovering a target entity from a polydisperse liquid using *co-flow* microfiltration and ultrafiltration processes, as recited in claims 1 and 28 of the instant application. The focus and emphasis of Kopf's process involves the use of a cross flow filtration module where there is a porous filter element across a surface of which liquid medium to be filtered is flowed in tangential flow fashion for permeation, through the filter element, of selected components of the liquid medium. *See* Kopf, column 3, lines 35-40. In contrast, in the coflow technique of the present application, the permeate is circulated in the shell side of the hollow fiber module in the same direction as the retentate resulting in similar pressure drops in the bore of the tube and shell side leading to approximately uniform transmembrane pressure in the axial direction of the hollow fiber membrane module. *See* page 19, lines 8-14 of the present application. Nowhere does Kopf suggest using the coflow technique. As discussed in paragraphs [0010] to [0013], use of the co-flow technique of the present invention is important in achieving the yields and purity of the present invention with a polydisperse, high fouling liquid like milk. In view of these distinctions, it is apparent that Kopf is not anticipatory.

Accordingly, the rejection of claims 1-2, 4, 7, 8, 11-17, 28-29, 31, 34-35, and 38-44 under 35 U.S.C. § 102(b) as anticipated by that reference is improper and should be withdrawn.

The rejection of claims 48-49, 52, and 54-57 under 35 U.S.C. § 102(b) as anticipated by Menon et al, "Effect of Ion Binding on Protein Transport through Ultrafiltration Membranes," *Biotechnology and Engineering* 63(3):298-307 (1999) ("Menon") is respectfully traversed.

Menon quantifies the effects of specific ions on protein transport through ultrafiltration membranes over a range of solution pH and salt concentrations. Menon demonstrates that at a pH above a protein's isoelectric point there is a decrease in the sieving coefficient (ratio of protein in filtrate to bulk solution) due to the repulsive electrostatic interaction between charged protein and the pore boundary. Menon further discloses the separation of two proteins (i.e. IgG and BSA) having different isoelectric points by adjusting the pH and ionic strength to favor the retention of one and flux of the other. Menon teaches

the use of a batch standard stirred cell filter membrane in which the protein solution is added directly to a cylinder containing the filter membrane. Menon fails to teach a co-flow ultrafiltration technique, as recited by claim 48 of the present invention.

Accordingly, Menon is not anticipatory and the rejection of claims 48-49, 52, and 54-57 under 35 U.S.C. § 102(b) should be withdrawn.

The rejection of claims 3 and 30 under 35 U.S.C. § 103(a) for obviousness over Kopf in view of U.S. Patent No. 6,824,679 to Dzengleski et al. ("Dzengleski") is respectfully traversed.

The PTO acknowledges that Kopf, as discussed above, fails to teach the use of a helical hollow fiber membrane module which produces Dean vortices, as recited in claims 3 and 30 of the present application. Dzengleski is cited as teaching these features. However, Dzengleski does not teach coflow filtration techniques and, therefore, fails to overcome the above deficiency of Kopf. Indeed, the use of cross-flow is mentioned at column 11, lines 18-19 of Dzengleski.

Since the combination of Kopf and Dzengleski fails to teach or suggest every claim limitation, the rejection of claims 3 and 30 for obviousness under 35 U.S.C. § 103(a) is improper and should be withdrawn.

The rejection of claims 6 and 33 under 35 U.S.C. § 103(a) for obviousness over Kopf is respectfully traversed. Claims 6 and 33 require a transmembrane pressure (TMP) difference of less than 2 psi. The PTO asserts that although Kopf teaches a TMP range from 2-15 psi, it would have been a matter of routine optimization to achieve the claimed result.

Given that Kopf teaches achieving suitable results with a TMP range of 2-15 psi, there would have been no motivation to modify Kopf to utilize a different TMP range. Moreover, claims 6 and 33 depend from claims 1 and 28, respectively, which require coflow filtration. Since Kopf teaches a completely different type of filtering and provides no basis to expect that results obtained with cross-flow filtration would be achieved with co-flow, the claimed subject matter would not have been obvious. Therefore, the rejection of claims 6 and 33 under 35 U.S.C. § 103(a) for obviousness over Kopf is improper and should be withdrawn.

The rejection of claims 5, 10, 21-26, 32, and 37 under 35 U.S.C. § 103(a) for obviousness over Kopf in view of Menon is respectfully traversed. The PTO acknowledges that Kopf does not discuss carrying out the microfiltration process at the target entity's

isoelectric pH, carrying the ultrafiltration process at a pH different than the target's isoelectric pH, using a pH above that at which the target entity precipitates, using a pH greater than 8.5, employing a pH greater than 10, or carrying out ultrafiltration at an ionic strength of 10-20 nM NaCl (or 12-17 nm NaCl). However, the PTO asserts that one of skill in the art would have been motivated to modify the method of Kopf by incorporating the teachings of Menon related to modifying solution pH and salt concentration to alter protein transport through ultrafiltration membranes.

Since Kopf and Menon teach completely different types of filtration - - i.e. cross-flow v. stirred cell, one or ordinary skill in the art would not have been motivated to combine these references, let alone expect that results achieved with one would be obtained with the other. Further, neither Kopf nor Menon teach a coflow microfiltration process nor coflow ultrafiltration process, as claimed. Since Kopf and Menon fail to teach or suggest every limitation of claims 5, 10, 21-26, 32, and 37, the rejection of these claims under 35 U.S.C. § 103(a) for obviousness is improper and should be withdrawn.

The rejection of claims 9 and 36 under 35 U.S.C. § 103(a) for obviousness over Kopf in view of Dzengleski and further in view of Menon is respectfully traversed for substantially the reasons noted above.

The rejection of claims 18-19 and 45-46 under 35 U.S.C. § 103(a) for obviousness over Kopf in view of U.S. Patent Application No. 2003/0033637 to Oishi et al. ("Oishi") is respectfully traversed. The PTO acknowledges that Kopf does not particularly describe using a cell culture fluid from transgenic plant cells. Oishi is cited as teaching this feature. However, since Oishi does not overcome the above-noted deficiencies of Kopf, the rejection of claims 18-19 and 45-46 for obviousness is improper and should be withdrawn.

The rejection of claims 20 and 47 under 35 U.S.C. § 103(a) for obviousness over Kopf in view of Luque et al., "A New Coiled Hollow Fiber Module Design for Enhanced Microfiltration Performance in Biotechnology," *Biotechnology and Bioengineering* 65(3):247-57 (1999) ("Luque") is respectfully traversed.

Luque describes subjecting various polydisperse liquids to microfiltration, and subsequently subjecting the microfiltration membrane to an acid-free cleaning regime. However, Luque fails to overcome the deficiencies of Kopf described above. Accordingly, this combination of references cannot be properly used to reject claims 20 and 47 and the rejection of them for obviousness should be withdrawn.

The rejection of claim 27 under 35 U.S.C. § 103(a) for obviousness over Kopf in view of U.S. Patent No. 5,204,002 to Belfort et al. ("Belfort") is respectfully traversed. Belfort is cited as teaching flux values between 100-130 lmh for the filter membrane, as recited by claim 27. Belfort, however, does not teach a coflow microfiltration system and therefore does not overcome the above-noted deficiencies of Kopf.

Accordingly, the rejection of claim 27 under 35 U.S.C. § 103(a) for obviousness is improper and should be withdrawn.

The rejection of claims 50 and 51 under 35 U.S.C. § 103(a) for obviousness over Menon is respectfully traversed. Menon teaches a completely different filtration procedure than the coflow process of the claimed invention and provides no expectation that results similar to those achieved with a stirred cell process would be obtained using coflow. Therefore, the subject matter of claim 50-51 would not have been obvious from Menon.

Accordingly the rejection for obviousness is improper and should be withdrawn.

The rejection of claim 53 under 35 U.S.C. § 103(a) for obviousness over Menon in view of Belfort is respectfully traversed for substantially the same reasons noted above.

The rejection of claims 58-60 under 35 U.S.C. § 103(a) for obviousness over Menon in view Kopf is respectfully traversed for substantially the same reasons noted above.

The rejection of claims 61-62 under 35 U.S.C. § 103(a) for obviousness over Menon in view of Oishi is respectfully traversed for substantially the reasons noted above.

In view of all of the foregoing, applicants submit that this case is in condition for allowance and such allowance is earnestly solicited.

Respectfully submitted,

Date: August 20, 2007

Michael L. Goldman
Registration No. 30,727

NIXON PEABODY LLP
Clinton Square, P.O. Box 31051
Rochester, New York 14603-1051
Telephone: (585) 263-1304
Facsimile: (585) 263-1600

Certificate of Mailing - 37 CFR 1.8(a)

I hereby certify that this correspondence is being deposited with the United States Postal Service as first class mail in an envelope addressed to: Commissioner for Patents P.O. Box 1450 Alexandria, VA 22313-1450, on the date below.

8/20/07 Wendy L. Barry

Date Wendy L. Barry